Behavioral Neuroscience
1991, Vol. 105, No. 3, 482-486

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0735-7044/91/$3.00

Radial Maze Learning Deficits and Mediodorsal Thalamic Damage in Context of Multifocal Seizure-Induced Brain Lesions

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Rats were either trained 21 days after seizure induction or trained before seizure induction and tested 21 days later. The seizures, which induce insidious brain damage within a multitude of diencephalic and subcortical telencephalic structures, were induced by a single injection of lithium (3 mEq/kg sc) followed 24 hr later by pilocarpine (30 mg/kg sc). Compared with controls, the treated rats displayed significant deficits in the acquisition and recall of a radial maze task. Although there was multifocal brain damage, only the amount of damage within the mediodorsal thalamic group was significantly and strongly correlated ($\rho = .79$) with numbers of errors.

Although the traditional approach to understanding the relationship between brain and behavior involves disruption of singular regions within the brain (e.g., by experimental lesions), clinical populations frequently display multifocal lesions that exhibit insidious properties. We have been interested in the effects on behavior of multifocal brain damage that slowly increases over time to more clearly understand (a) the potential interactions between compromised brain regions and (b) how multifocal damage might affect the magnitude and perhaps even the qualitative characteristics of behaviors that dominate an animal's profile but then become less frequent.

Honchar, Olney, and Sherman (1983) found that rats injected with (a) a single systemic dosage of lithium (3 mEq/kg) and (b) a single systemic amount of pilocarpine (30 mg/kg) 24 hr later displayed limbic seizures within 30 min of the second injection. The seizures were similar to the Stage IV kindling display and comparable to those generated by 300 mg/kg of pilocarpine alone (Turski et al., 1983). Histopathological analyses have shown that insidious alterations in brain morphology (Persinger, Makarec, & Bradley, 1988) continues to occur for at least 100 days within the brains of these animals, even though casual observations reveal no interictal behavioral anomalies.

Two conspicuous types of histological changes have been noted; their magnitudes achieve asymptote approximately 3 weeks after seizure onset (Peredery, 1989). Neuronal (soma) necrosis and gliosis are evident within two major regions: the limbic cortices (entorhinal to the insular areas, inclusive) and the thalamus. Within the thalamus, pervasive damage is noted within the mediodorsal (MD) thalamic group and the posterior group; singular nuclear groups, such as the suprageniculate nucleus are often conspicuously damaged. The hippocampus is only mildly affected and hypothalamic changes have never been observed; neocortical damage is marginal. Mild gliosis is evident in portions of the basal ganglia. The mesencephalon, with the exception of near-complete devastation of the reticulata portion of the substantia nigra, is preserved.

One to two months after the induction of seizures by the injections of the lithium and pilocarpine, rats display a marked reduction in conditioned taste aversion (Venugopal & Persinger, 1988), the magnitude of which is coupled to the amount of damage within the insular cortices and thalamic gustatory nucleus. Unpublished experiments by Harrigan (1989) demonstrated that lithium–pilocarpine-treated rats were still displaying occasional spontaneous seizures several months after the single pair of injections. The interresponse times (IRTs) of normal rats reflected the differential rewarding of low rates of responding interval (6 and 12 s), whereas IRTs of the rats that had seizures clustered around 1–2 s; their inhibitory or timing capacity had been markedly and effectively compromised (Harrigan, Peredery, & Persinger, 1990).

We decided to investigate the specific and quantitative relationships between the behavior and the pattern of brain damage induced by the seizures that are evoked by a single lithium–pilocarpine injection. A radial maze task (Olton, Becker, & Handelmann, 1979) was selected for behavioral testing because of its sensitivity in detecting lesion-induced damage within the MD nucleus of the thalamus, the amygdaloid region (Raffaele & Olton, 1988), and the hippocampus (Beatty & Troster, 1988; Stokes & Best, 1988).

Method

Subjects

Fifteen 90–110 day old male Wistar albino rats were used as subjects.

We thank Ayerst Laboratories, Montreal, Quebec, Canada, for supplying the acepromazine.

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Table 1
Means and Standard Deviations (M ± SD) for the Number of Errors and Time Required (in Minutes) to Complete the Eight-Armed Radial Maze Task for Days 1–2 (D1–D2), Days 7–8 (D7–D8), and the Total 8 Days of Training or Testing

		Errors			Time		
Group	*n*	D1–D2	D7–D8	D1–D8	D1–D2	D7–D8	D1–D8
Pretrain							
Exp	4	4.0 ± 1.1	1.6 ± 1.4	2.4 ± 1.0	6.3 ± 1.6	4.6 ± 2.9	4.9 ± 1.0
Con	4	6.7 ± 2.0	2.1 ± 1.7	3.9 ± 1.3	8.1 ± 1.1	6.0 ± 2.8	6.6 ± 1.3
Posttest							
Exp	4	14.4 ± 7.7	13.2 ± 6.6	12.1 ± 3.3	11.8 ± 2.3	6.9 ± 3.9	8.9 ± 1.0
Con	4	3.5 ± 0.7	1.0 ± 1.6	2.6 ± 1.7	3.1 ± 0.9	2.5 ± 0.7	2.8 ± 0.7
Posttrain							
Exp	5	14.6 ± 7.1	13.7 ± 4.4	13.8 ± 3.8	12.4 ± 1.7	3.7 ± 1.3	7.6 ± 1.7
Con	2	6.8 ± 0.3	0.8 ± 1.0	3.5 ± 1.0	8.2 ± 1.0	2.5 ± 2.1	5.5 ± 0.7

Note. Pretrain = preseizure training group (Experiment 1); Posttest = postseizure testing group (Experiment 1); Posttrain = postseizure training group (Experiment 2); Exp = treated animals; Con = control animals.

Procedure

Seizure and recovery. Twenty-four hours after they received 3 mEq/kg lithium chloride subcutaneously, 9 rats received 30 mg/kg pilocarpine through the same route. Within about 30 min, all rats displayed forepaw clonus and rearing, as reported by Honchar et al. (1983). To ensure survival, rats also received 25 mg/kg (10 mg/cc) acepromazine (Atravet, Ayerst Labs., Montreal, Quebec, Canada). After about 5–10 days of aphagia and adipsia, except for a preference for rat chow mush in water (or milk) in a bowl, the rats began to access the food hopper and water bottles. Both the seizure and control rats were housed in single cages; food and water were available ad libitum.

Shaping and training. Rats were allocated to one of four groups: *preseizure trained* (n = 4), *postseizure trained* (n = 5), *preseizure controls* (n = 4), and *postseizure controls* (n = 2). Before training, the rats were reduced to 85% of their free-feeding body weights over a 6 day period. They were then habituated to an eight-arm radial maze (RAM) for 15 min per day for 3 days. Each arm of the maze (80 cm from the floor) was 101.5 cm long and 10.5 cm wide; the diameter of the central arena was 66.0 cm. During the habituation period, rats were shaped to run to the end of each arm; this was completed by moving the rewards (two 45-mg Noyes pellets) on successive days (sessions) from the entry to the arms, then to the midportion of each arm, and finally to the end of each arm. On all consequent days, the rats were required to run to the end of each arm to obtain reward.

The numbers of errors (return to an arm that had been entered previously) and the time required to obtain the rewards from all eight arms (truncated at 15 min) were recorded for eight consecutive daily sessions. This period was selected because previous work in our laboratory indicated that most rats reach asymptote in both errors and maze time after 8 days of experience. When each rat had completed its session, the arms and the central arena ("start" area) were washed with 0.4% acetic acid and air dried.

Group testing. After 8 days of training in the RAM, rats in the preseizure training group were injected with lithium–pilocarpine. After recovery 21 days later, these rats and their controls (Experiment 1) were again given 8 days of testing within the RAM. Approximately 21 days after the lithium–pilocarpine injections, the postseizure group and its controls (Experiment 2) were trained (after 3 days of maze habituation) for 8 days on the RAM.

Histology and measures of histopathology. One to two days after the RAM testing (Experiment 1) or training (Experiment 2) had been completed (about 30–34 days after induction of the seizures), the rat brains were removed, fixed in an ethanol–formalin–acetic acid solution, processed, and embedded in paraffin. Every twentieth 10-μm coronal section between the caudal mesencephalon and the level of the anterior commissure was stained with thionin (Persinger, 1980). All structures that are listed in Paxinos and Watson (1986) were examined (magnification = ×100 or ×400) by an experienced histopathologist who was not familiar with the treatments of the animals. A score for histological damage, if present, was determined. The scores ranged from 1 to 7 and reflected a continuum of neuronal drop out, gliosis, and the accumulation of dense Nissl-staining material (probably cell fragments). All structures that displayed damage were then aggregated into 14 major clusters; they reflected traditional organizations. Because no damage was ever observed in the hypothalamus, neocortex, ventral forebrain, or mesencephalic structures (except the reticulata of the substantia nigra), these regions were not included in the analyses.

Results

The means and standard deviations for the mean number of errors and mean time (in minutes) to complete the RAM task for the first 2 days, the last 2 days, and all 8 days for the experimental and control groups of Experiments 1 and 2 are shown in Table 1. A one-way analysis of variance demonstrated no significant group differences (preseizure vs. controls) for Experiment 1 in either maze time or errors before the induction of seizures. However, during the testing period the seizure group displayed significantly more errors, $F(1, 6) = 24.95$, $p = .002$, and longer maze time to reach the criterion, $F(1, 6) = 45.03$, $p < .001$, than the controls. A multivariate analysis of variance (MANOVA) for the mean errors and maze time for the first two and last two sessions for the training and testing periods verified the strong Group × Pre–Postseizure interaction, Fs(1, 6) = 65.74 and 90.35, respectively, ps < .001. Repeated measure analyses also indicated a significant Group × Pre–Postseizure interaction, $F(1, 6) = 10.88$, $p < .01$, for body weights for the control

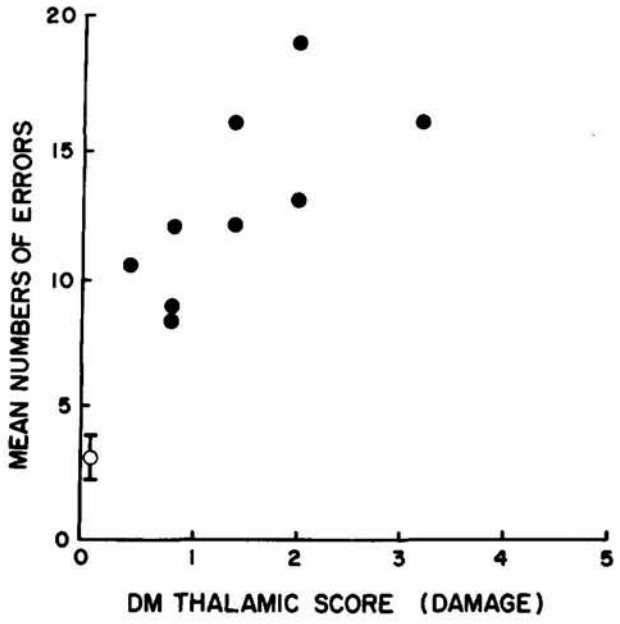

Figure 1. Mean number of errors displayed per day within the radial maze as a function of the severity of histopathology (primarily neuronal drop out and gliosis) for rats that were trained or tested about 3 weeks after the induction of limbic seizures by lithium–pilocarpine. (Open circle represents the control group scores; vertical brackets indicated 1 *SD*.)

Table 2
Means and Standard Deviations for the Histopathology Scores for Each Major Cluster of Structures That Displayed Damage

Group	*M*	*SD*
Lateral thalamus	1.76	0.64
Mediodorsal thalamus	1.42	0.87
Limbic cortex	1.37	0.95
Intralaminar thalamus	1.36	0.92
Posterior thalamus	1.27	0.30
Corticoamygdaloid region	1.00	0.96
Midline thalamus	0.73	0.50
Metathalamus	0.68	0.49
Nigrostriatal complex	0.56	0.31
Hippocampus	0.46	0.11
Lateral amygdala	0.39	0.33
Basal amygdala	0.39	0.30
Anterior thalamus	0.31	0.48

(388 ± 19, 418 ± 17) and seizure (361 ± 21, 350 ± 37) groups.

There were no statistically significant differences in either mean errors or maze time between the 5 rats that had seizures before training and the 4 rats that had seizures after training. A MANOVA indicated that both seizure groups displayed a significant reduction in maze time (no Group × Time interaction) between the first two and last two sessions, $F(1, 7)$

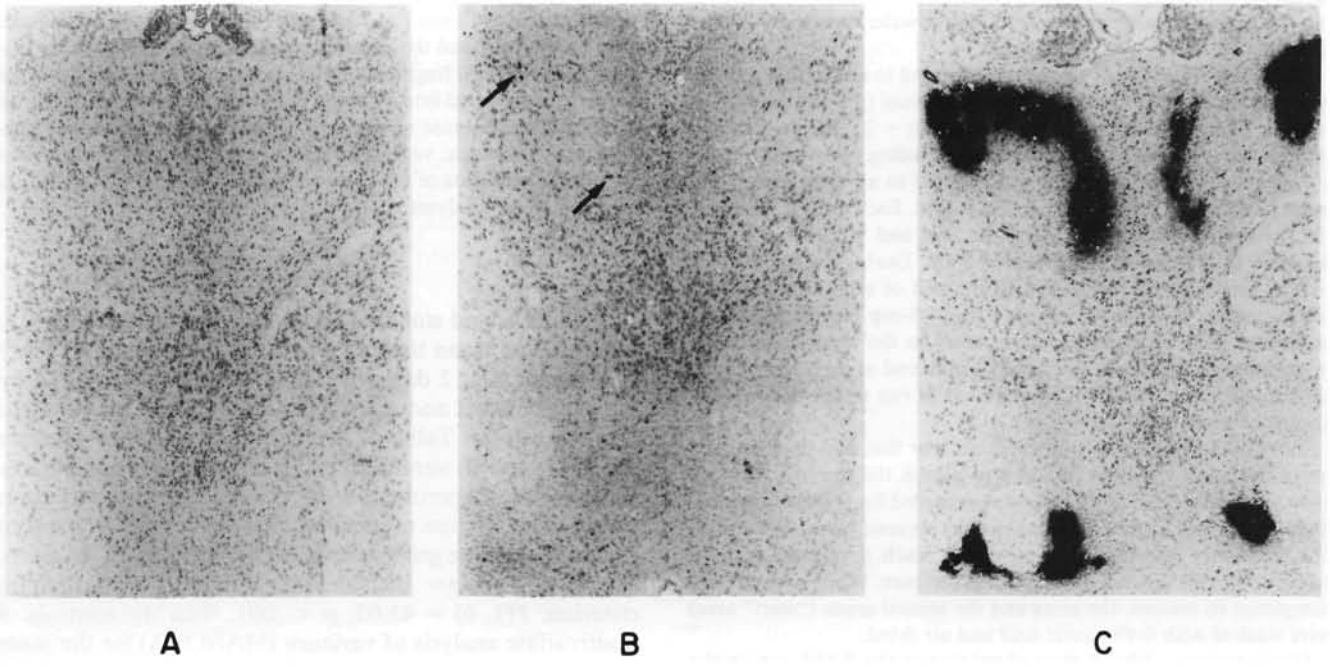

Figure 2. Photomicrographs (×74) of coronal sections at the level of the mediodorsal nucleus (MD) for a control rat (A), a rat that displayed low to moderate necrosis within the MD after seizure induction by a single subcutaneous injection of lithium and pilocarpine (B), and a rat that displayed extreme necrosis within the MD about 35 days after the treatment (C). (Deep-staining material is periodic acid Schiff positive and calcium reactive.)

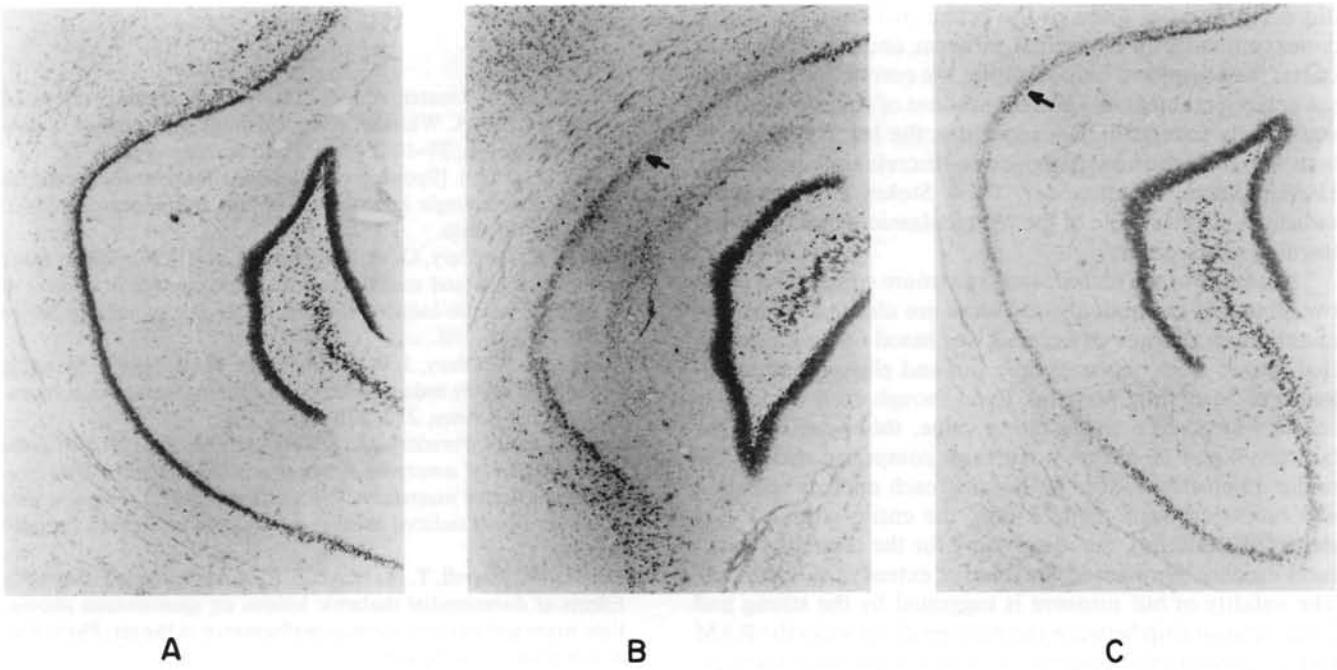

Figure 3. Photomicrographs (×74) of coronal sections of the Ammon's horns region of the hippocampus for a control rat (A) and two rats (B and C) that had received the seizure-inducing subcutaneous lithium and pilocarpine injections before testing in the radial maze. (Arrows indicate the region [primarily CA1] of the most severe neuronal loss.)

= 30.02, $p = .001$, but no significant change in numbers of errors, $F(1, 7) = 0.11$, $p > .05$. There were no significant differences in mean errors or maze time between the 4 controls from Experiment 1 and the 2 controls from Experiment 2. Although body weights for the rats that were trained after the seizure were significantly greater than those trained before the seizure, the former did not differ significantly from the controls.

The means and standard deviations for the histopathological scores for each of the nuclear clusters within the diencephalon and subcortical telencephalon are shown in Table 2. Analyses (t tests) indicated no significant differences between the groups that had seizures before or after the training; consequently they were combined ($n = 9$). Spearman ρ and Pearson r correlations were completed between the histopathological score for each group of structures and the mean number of errors and maze time for the testing (Experiment 1) and training (Experiment 2) periods after the seizure. The only statistically significant correlation (Figure 1) occurred between the amount of histopathological damage within the MD thalamic group and the mean number of errors ($\rho = .79$, $r = .70$, $p < .01$) over the eight daily sessions but not the mean time within the RAM ($\rho = -.27$).

Figure 2 illustrates the severity of the necrosis within the MD thalamus for a treated rat that displayed mild to moderate damage (Figure 2B) and a rat that displayed extreme damage (Figure 2C); the same region for a control rat (Figure 2A) is shown for comparison. The dark basophilic material in Figure 2C has been shown by atomic absorption analyses and histochemical staining to contain significant concentrations of calcium, which is embedded within a dominant neutral mucopolysaccharide that is reactive to periodic acid Schiff but not to Alcian blue (Lafreniere, Peredery, & Persinger, 1990). This material has been observed only in treated rats. Between 15 and 50 days after the lithium–pilocarpine treatment, the material changes from an amorphous material to a crystalline form.

For comparison, sections of Ammons' horns region of the hippocampus from 2 treated rats (Figures 3B and 3C) and 1 control rat (Figure 3A) are shown in Figure 3. Cell loss for rats in which the lithium–pilocarpine seizures had been induced was primarily in the CA1 region of the hippocampus. Necrotic estimates, based on the number of successive slides that displayed discriminable neuronal drop out, were not correlated with the number of errors in the RAM task. The dark-staining material was never evident within the hippocampus or any of the limbic (entorhinal) cortices.

Discussion

The results of the present study strongly suggest that the amount of seizure-induced damage to the MD portion of the thalamus was responsible for deficits in both the learning and the retention of the RAM task. Although significant damage

did occur in other areas of the brain, including the limbic cortex (primarily the entorhinal, piriform, and insular regions), lateral thalamus, and hippocampus, the correlations between the behavioral measures and the amount of damage were not statistically significant. Consequently, the behavioral correlates of both multifocal damage and discrete lesions (Means, Harrell, Mayo, & Alexander, 1974; Stokes & Best, 1988) indicate a primary role of the MD thalamic group in spatial learning and memory.

The results of the present study are more compelling when two potential methodological issues are clarified. First, the quantitative measure of necrosis was based on a rating scale that ranged from neuronal drop out and gliosis to accumulation of basophilic material. Even though each nucleus or region was given a single rating value, the mean score for between 4 and 10 of these structures comprised each of the major 13 clusters (Table 2). Because each nucleus or region was homogeneously affected (i.e., the entire structure was damaged similarly), the mean score for the cluster of structures crudely represented the relative extent of the necrosis. The validity of our measure is suggested by the strong and linear relationship between the number of errors in the RAM task and the computer-determined score for brain damage. Because the histopathologist was not familiar with the behavioral history of the rat, the reliability of this method is further supported.

The second potential issue involves the possibility that the statistically significant correlation between the number of errors in the RAM task and the amount of MD damage was a chance artifact of the 13 correlations. However, the strength of the correlation (using both quantitative ranges and rank order criteria) reduces the likelihood that chance was operating. We emphasize that none of the correlations between either time to criterion or errors in the RAM task and necrotic score for the other brain regions even approached statistical significance.

A more recent study (Persinger, Bureau, Kostakos, & Peredery, 1990) has replicated the results that rats in which limbic seizures were induced by a single injection of lithium–pilocarpine 50 days before testing display significantly more RAM errors than do controls. The effect was dose dependent, and there was no significant increase in either errors or time to criterion on days when treated rats either displayed rearing and forepaw clonus or did not display these seizures while being tested. However, the absence of the latter differences does not exclude the possibility that subclinical seizures were occurring because we did not directly measure brain electrical activity. Histological analyses were not performed.

Additional experiments with other rats (Persinger et al., 1990) involved the assessment of reference and working memory. Rats in which seizures had been induced 30–50 days before testing by the same dosages of lithium and pilocarpine that were used in the present study displayed severe deficits in both types of memory. Although the brains of these rats were not examined, both their quantitative error rates and the characteristics of their learning curves were very similar to those displayed by rats in which the MD nucleus had been destroyed by injections of ibotenic acid (Stokes & Best, 1990).

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Received November 17, 1989
Revision received August 21, 1990
Accepted December 21, 1990 ■